Carbon-13/Carbon-12 Ratio Variability in the Genus *Lilium*

LANDIS W. DONER AND MARVIN P. THOMPSON
Eastern Regional Research Center[1]
Philadelphia, Pennsylvania 19118, USA

Key words: Carbon-13/carbon-12 ratios—*Lilium*

We have surveyed $\delta^{13}C$ values within a given plant genus, *Lilium*, to gain some insight into factors responsible for interspecies variations. *Lilium* species (C_3 photosynthetic pathway) native to North America were significantly lighter in ^{13}C than those native to Europe and Asia, regardless of where they were grown. Interspecies variations in the ability to facilitate diffusion of CO_2 to carboxylation sites in leaf mesophyll, rather than environmental factors, probably are responsible for the degrees to which C_3 plants discriminate against $^{13}CO_2$.

Introduction

The primary characteristic which determines the extent to which a plant discriminates against $^{13}CO_2$ during photosynthesis is its photosynthetic category [1, 8, 9]. C_3 plants possess $\delta^{13}C$ values [per mil variations of $^{13}C/^{12}C$ ratios relative to Pee Dee belemnite (PDB) limestone standard] ranging from -22 to $-34°/_{00}$, while C_4 plants possess values ranging from -10 to $-18°/_{00}$. Crassulacean acid metabolism (CAM) plants possess a wide range of intermediate $\delta^{13}C$ values. While the main differences in $\delta^{13}C$ values between C_3 and C_4 plants are known to result from the high kinetic isotope effect displayed by ribulose bisphosphate carboxylase in C_3 plants, the reasons for the range of values within each photosynthetic category are not known.

A comprehensive review [6] of factors involved in carbon isotope fractionation by plants has shown that ribulose bisphosphate carboxylase exhibits a kinetic isotope effect of -20 to $-40°/_{00}$ on atmospheric CO_2 ($\delta^{13}C = -6.4$ to $-7.0°/_{00}$). Phosphoenolpyruvate carboxylase, the initial CO_2 trapping enzyme in C_4 plants, shows little discrimination. Subsequent transfer of the fixed CO_2 to ribulose bisphosphate carboxylase in C_4 plants is quantitative, so in these plants the enzyme does not fractionate isotopes in CO_2. Since wide-ranging $\delta^{13}C$ values exist within C_3 and C_4 plant categories, obviously there are factors affecting these values in addition to differential isotope effects of the enzymes involved. Broad surveys of $\delta^{13}C$ values in several species have been carried out, and those with Atriplex (may possess either C_3 or C_4 pathway) have been most informative in attempts to understand natural variations in $\delta^{13}C$; results have been reviewed [6, 9]. Quantitative expressions have been developed, most recently by O'Leary [6] and Farquhar et al. [5], from which $\delta^{13}C$ values can be predicted when variables such as CO_2 concentrations in the atmosphere surrounding the leaf (*Ca*) and in leaf intercellular spaces (*Ci*) are quantified. A useful expression [5] for C_3 plants is

$$\delta^{13}C \text{ plant} = \delta^{13}C \text{ atmosphere} - a - (b\text{-}a)\, Ci/Ca$$

[1]Agricultural Research Service, U.S. Department of Agriculture.

where the constant a is the diffusivity of $^{12}CO_2$ relative to $^{13}CO_2$ (4.4°/₀₀) and b (assumed constant) is the isotopic discrimination by ribulose bisphosphate carboxylase (approx. 30°/₀₀). From the above equation, it is clear that variations in *Ci/Ca* will modify $\delta^{13}C$ plant. Effects limiting the capacity of leaf mesophyll to photosynthesize (such as low light intensity) will increase *Ci/Ca* and reduce $\delta^{13}C$ plant; *Ci/Ca* will be reduced by factors which reduce the assimilation rate of CO_2 through the stomata, and $\delta^{13}C$ plant will be increased [5]. Measurements of $\delta^{13}C$ in plants grown under controlled environmental conditions support the use of the above equation [4]. It is well known that numbers and dimensions of stomata influence photosynthetic rates, and evidence indicates [10] that stomatal aperture is determined by the capacity of plant tissue to fix CO_2.

Materials and methods

Leaf samples of *Lilium* species were solicited from individual growers in Minnesota, Oregon, and Pennsylvania. In most instances, one leaf specimen was collected for each species although two or more were collected for some species. The $\delta^{13}C$ values were determined after combustion of leaf tissue to CO_2 by Coastal Science Laboratories[2], Port Aransas, TX, USA. A Micromass 602D mass spectrometer (VG-Isotopes Ltd., Winford, Cheshire, UK) was used, and general procedures, including combustion methods and corrections applied, were as described in Doner and White [2]. Overall accuracy for $^{13}C/^{12}C$ determination including combustion and mass spectrometry, is 0.3°/₀₀ or better.

Results and discussion

Lilium species native to North America or Europe and Asia were analyzed for $\delta^{13}C$ to determine if differences occur when they are grown in the same location under identical conditions. The genus *Lilium* was selected because it consists of fewer than 90 species which possess differences in morphology and growth characteristics. We determined $\delta^{13}C$ values for leaf tissue from mature plants representing 43 distinct species (see Tables 1, 2). The range in $\delta^{13}C$ for all samples is 6.3°/₀₀, to our knowledge the widest yet encountered for a given genus. Table 3 presents a statistical summary of the results, as well as data for plants which were grown in the same environment. Species native to North America are significantly ($p < 0.01$) lighter in ^{13}C than those species native to Europe and Asia; plants grown under identical conditions of time and environment (in Oregon) show this contrast even more markedly. Therefore, variations in physiology and physiognomy rather than environmental factors seem to determine interspecies variations in $\delta^{13}C$ values. Possibly these variations result in different rates of diffusion of CO_2 from the atmosphere to the leaf intercellular spaces. In comparing $\delta^{13}C$ values for species of *Lilium* which are closely related taxonomically [3], we found variations up to 3.4°/₀₀. Coincidentally, we have observed that there is a significant ($p < 0.01$) correlation ($r = 0.74$) between $\delta^{13}C$ value and number of guard cells (and hence stomata) per unit area of leaf surface. Twenty-one samples were examined, and it was observed that the fewer the number of guard cells, the more positive $\delta^{13}C$ generally becomes (Figure 1). This observation supports the models [6, 5] wherein factors in C_3 plants resulting in resistance to CO_2 diffusion will reduce *Ci/Ca* and cause $\delta^{13}C$ values to become more

positive. Those data points which fall below the curve (dark circles) represent the European (Caucasian) groups of *Lilium*.

The results presented here suggest that for the genus *Lilium*, variations in form between North American and European and Asian species may translate into differential effects upon diffusion of CO_2 in these plants. This may explain why differences exist in $\delta^{13}C$ values among lilies native to different areas.

Acknowledgements

We thank Dr. John G. Phillips, Consulting Statistician, Northeastern Region, Agricultural Research Service, U.S. Department of Agriculture, for statistical counsel; Edward and Judith McRae (Boring, OR, USA); Hugh Cocker (Rochester, MN, USA); and Josephine de N. Henry (Gladwyne, PA, USA) for leaf samples of *Lilium* species.

References

1. Bender MM (1971) Variations in the $^{13}C/^{12}C$ ratios of plants in relation to photosynthetic carbon dioxide fixation. Phytochemistry 10:1239-1244
2. Doner LW and White JW, Jr. (1977) Carbon-13/carbon-12 ratio is relatively uniform among honeys. Science 197:891-892
3. Feldmaier C Lilies (1970) BT Batsford, London, UK
4. Farquhar GD, Ball MC, von Caemmerer S and Roksandie Z (1982b) Effect of salinity and humidity on $\delta^{13}C$ value of halophytes—evidence for diffusional isotope fractionation determined by the ratio of intercellular atmospheric partial pressure of CO_2 under different environmental conditions. Oecologia (Berl.) 52:121-124
5. Farquhar GD, O'Leary MH and Berry JA (1982a) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121-137
6. O'Leary M (1981) Carbon isotope fractionation in plants. Phytochem 20:553-567
7. Roane M and Henry J (1980) Notes on four lilies from Southeastern United States. Quart Bull, No Amer Lily Soc 34:25-32
8. Smith BN and Epstein S (1971) Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant Physiol 47:380-384
9. Troughton JH (1972) Carbon isotope fractionation by plants. Proc 8th Int Congr on Radiocarbon. The Royal Society of New Zealand 420-437
10. Wong SC, Cowan IR and Farquhar GD (1979) Stomatal conductance correlates with photosynthetic capacity. Nature 282:424-426

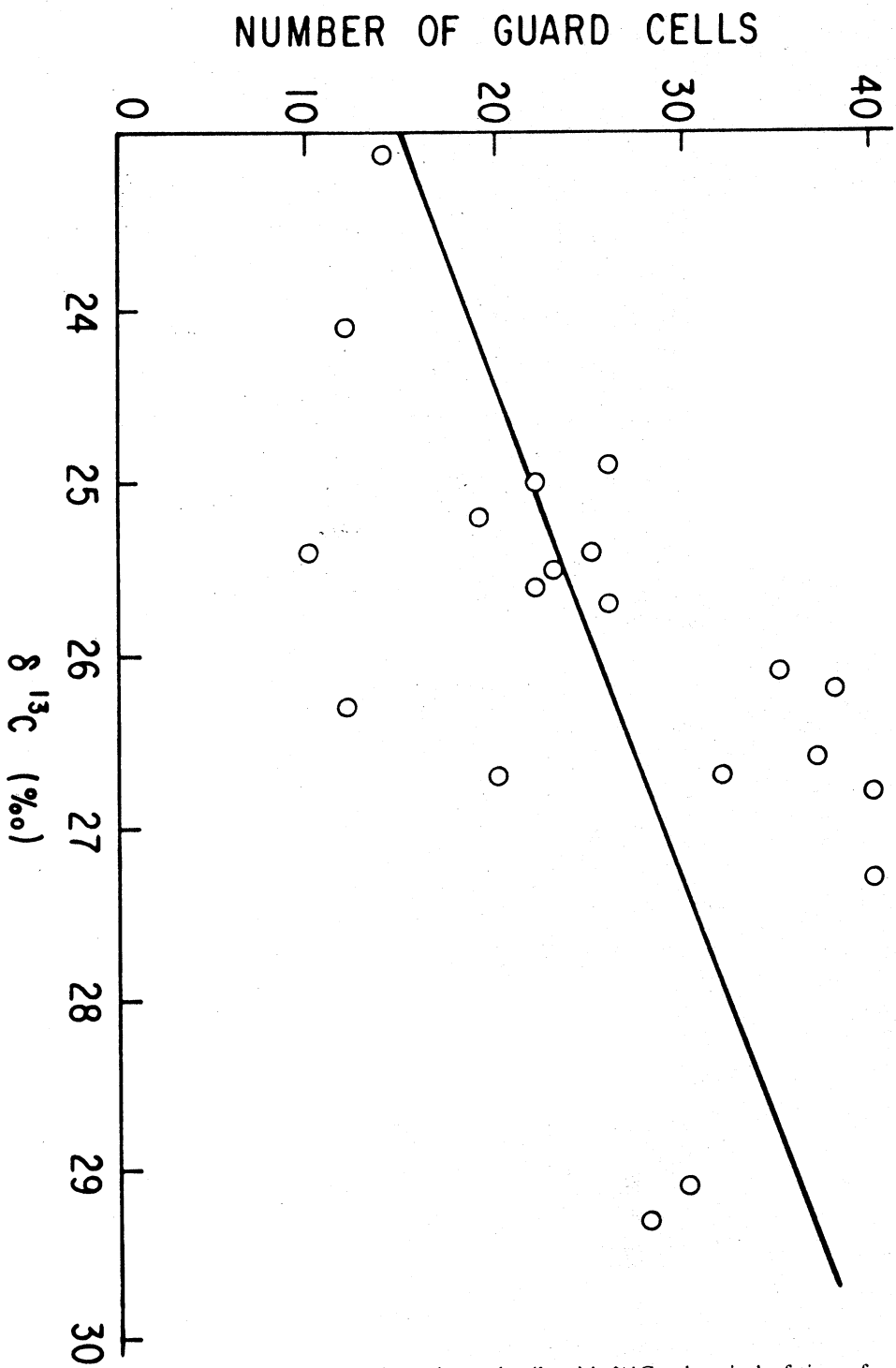

Figure 1. Relationship between number of guard cells with $\delta^{13}C$ values in leaf tissue from species of the genus *Lilium*

TABLE 1. Stable carbon isotope ratios of North American species [3] of the genus *Lilium*.

	Eastern	
1.	*L. canadense* var *editorum*	−27.3
2.	*L. canadense* var *flaviforum*	−26.2
3.	*L. fortuno fulgidum*[a]	−29.1
4.	*L. gazarubrum*[a]	−25.5
5.	*L. grayi*	−27.6
6.	*L. iridollae*	−25.2
7.	*L. mary henryae*[a]	−29.3
8.	*L. michauxii*	−25.7
9.	*L. michiganense*	−26.2
10.	*L. superbum*	−25.6
	Western	
11.	*L. columbianum*	−28.3
12.	*L. kelloggii*	−27.7
13.	*L. pardalinum*	−28.1
14.	*L. washingtonianum*	−27.2

[a]Proposed as new species [7].

TABLE 2. Stable carbon isotope ratios of Asian and European species [3] of the genus *Lilium*.

	Asian	
15.	*L. alexandrae*	−25.4
16.	*L. auratum* var *platyphyllum*	−25.5
17.	*L.* black beauty (hybrid)	−24.1
18.	*L. brownii*	−26.1
19.	*L. concolor*	−26.7
20.	*L. davidii* var *concolor*	−27.7
21.	*L. henryi* (type)	−26.3
22.	*L. henryi* var *flaviforum*	−23.0
23.	*L. japonicum*	−28.9
24.	*L. lankongense*	−26.1
25.	*L. leichtlinii*	−27.8
26.	*L. leucanthum centifolium*	−24.8
27.	*L. longiflorum* var *Ace*	−27.3
28.	*L. longiflorum* var *Ace*	−25.0
29.	*L. maximowicii* var *unicolor*	−25.6
30.	*L. nepalense*	−28.1
31.	*L. nobilissium*	−27.1
32.	*L. polyphyllum*	−29.0
33.	*L. pumilum*	−25.4
34.	*L. regale*	−25.5
35.	*L. rubellum*	−27.3
36.	*L. speciosum*	−26.8
37.	*L. szovitsianum*	−25.4
38.	*L. tigrinum* (type)	−25.8
39.	*L. tigrinum* var *flaviflorum*[a]	−24.7
40.	*L. tsingtauense*	−25.1
41.	*L. wallichianum*	−26.6
42.	*L. wardii*	−25.0
43.	*L. wilsonii*	−25.5
	European	
44.	*L. candidum*	−23.1
45.	*L. chalcedonicum*	−26.3
46.	*L. martagon* var *cattaniae*	−24.9
47.	*L. martagon* var *album*	−25.0
48.	*L. monadelphum*	−26.7

[a]May be a hybrid rather than a variant form.

TABLE 3. Statistical summary of $\delta^{13}C$ values of *Lilium* plants native to North America or Europe and Asia.[a]

				Grown in Oregon		
	North American	European and Asian	All samples	North American	European and Asian	All samples
No. samples	14	34	48	4	19	23
Range	−25.2 to −29.3	−23.0 to −29.0	−23.0 to −29.3	−27.2 to −28.3	−24.7 to −28.9	−24.7 to −28.9
Mean	−27.1	−26.0	−26.3	−27.8	−25.2	−25.7
SD	1.357	1.413	1.469	0.486	1.107	1.187

[a] *Lilium* plants other than these grown under identical conditions in Oregon were grown at various locations in the United States.